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The tetravalent meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine is immunogenic with a clinically acceptable safety profile in subjects previously vaccinated with a tetravalent polysaccharide vaccine

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SUMMARY

Objectives: The immunogenicity and safety of the tetravalent meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine (MenACWY–TT) were evaluated in subjects previously vaccinated with a tetravalent meningococcal polysaccharide vaccine and in subjects without previous meningococcal vaccination.

Methods: In this phase II, open, controlled study (NCT00661557), healthy subjects aged 4.5–34 years received one dose of MenACWY–TT at month 0. Subjects in the MPS group ($n = 192$) had received polysaccharide vaccine in a study conducted 30–42 months earlier; age-matched subjects in the noMPS control group ($n = 79$) had received no meningococcal vaccination within the past 10 years. Serum bactericidal activity using rabbit complement (rSBA) was measured at month 0 and month 1.

Results: At month 1, ≥97.0% of subjects had rSBA titers ≥1:128. Post-vaccination rSBA geometric mean titers (GMTs) were ≥3.9-fold higher than pre-vaccination in both treatment groups. Exploratory analyses showed no statistically significant differences between groups in percentages of subjects with rSBA titers ≥1:8 and ≥1:128, but significantly lower rSBA GMTs and vaccine response rates for each serogroup in the MPS versus the noMPS group. MenACWY–TT had an acceptable safety profile in both groups.

Conclusions: These results suggest that MenACWY–TT could be used in vaccination programs irrespective of the pre-vaccination status with polysaccharide vaccine.

1. Introduction

Neisseria meningitidis remains a major cause of invasive meningococcal disease and is responsible for at least 500 000 cases and 50 000 deaths each year.[1] *N. meningitidis* has been classified into 13 serogroups according to differences in capsular polysaccharides, of which serogroups A, B, C, W-135, and Y are responsible for most meningococcal disease cases.[2] The geographic distribution of the different serogroups differs and their epidemic capabilities are unpredictable.[2] Serogroups B and C are responsible for the majority of meningococcal disease cases in the USA and in Europe. Also, serogroup Y emerged as an important cause of meningococcal disease in the USA in the 1990s,[2] and this serogroup appears to be emerging in Scandinavian countries[3–5] and in the UK.[6] In Africa, Asia, and the Middle East, serogroup A is the predominant cause of meningococcal disease, and this serogroup has been responsible for large epidemics in the African meningitis belt.[2] Serogroup W-135 has also caused two large outbreaks, one in Saudi Arabia during the Hajj in 2000 and the other in Burkina Faso between 2002 and 2005.[7,8]

Many of the Hajj pilgrims to Saudi Arabia had been vaccinated with a bivalent polysaccharide vaccine against serogroups A and C before the outbreak caused by serogroup W-135 in 2000.[7] In 2002, in response to this outbreak, Saudi Arabia extended the recommendation from vaccination with the bivalent polysaccharide vaccine to vaccination with a tetravalent polysaccharide vaccine against serogroups A, C, W-135, and Y for all pilgrims. Indeed, vaccination remains the best strategy to prevent meningococcal disease, and polysaccharide vaccines are well tolerated, immunogenic, and provide protection for 3–5 years in adults and

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in children over the age of 2 years.[1] However, these vaccines result in the incomplete maturation of B-cells, do not induce immunologic memory, do not confer herd protection, and induce insufficient responses in children younger than 2 years of age, an age group at high risk for meningococcal infections.[9,10]

To overcome these limitations, capsular polysaccharides have been covalently coupled to carrier proteins to improve their immunogenicity and to induce a T-cell-dependent immune response.[9] Since 1999, meningococcal conjugate vaccines against serogroup C have been available and widely used.[11,12] Recently, a new serogroup A meningococcal conjugate vaccine has been successfully introduced in Africa.[13] Presently, two tetravalent meningococcal conjugate vaccines against serogroups A, C, W-135, and Y are licensed in various countries.[14,15] In addition, a new tetravalent meningococcal serogroups A, C, W-135, and Y tetanus toxoid conjugate vaccine (MenACWY–TT, *Nimenrix*™, GlaxoSmithKline Biologicals SA) is licensed for use in Europe as a single dose in individuals from the age of 2 years, and has been shown to elicit a good immune response and to have a clinically acceptable safety profile in toddlers, children, and young adults.[16–22] One potential advantage of conjugate vaccines over polysaccharide vaccines is that they prime for immunologic memory, which means that the vaccine should elicit higher responses to subsequent doses of polysaccharide or conjugate vaccines.[9,23] In contrast, polysaccharide vaccines may induce hyporesponsiveness to subsequent vaccination, in particular for serogroup C, although the clinical relevance of this phenomenon is unknown.[9,24–26]

The purpose of this study was to evaluate the safety and immune response induced by one dose of MenACWY–TT when given approximately 3 years after administration of a licensed tetravalent meningococcal plain polysaccharide vaccine (Men-PS).

2. Materials and methods

2.1. Study design

This was a phase II, open, controlled, single-center study (NCT00661557) conducted in Lebanon between May and December 2008, which was an extension of a previous trial (NCT00227422). Subjects were divided into three age strata: 4.5–10 years, 11–17 years, and 18–34 years of age at the time of vaccination in the present study. Within each age stratum, subjects were assigned 3:1 to two parallel vaccine groups: the MPS group included subjects who had received one dose of Men-PS in the study conducted 30–42 months earlier (NCT00227422) and the noMPS group included age-matched subjects who had not received any meningococcal vaccine within the past 10 years. The previous study was conducted in 2005 and was designed to evaluate the immunogenicity and safety of the Men-PS vaccine. In the present study, one dose of MenACWY–TT was administered to all the subjects in the MPS and the noMPS groups. Two blood samples were collected from all subjects for the analysis of the immune response to the MenACWY–TT vaccine, one prior to conjugate vaccination (month 0) and one at 1 month after conjugate vaccination (month 1). To determine safety, the patients were followed-up for 6 months after vaccination.

The study was conducted in accordance with good clinical practice guidelines and all applicable regulatory requirements, including the Declaration of Helsinki. Written informed consent was obtained from every subject, or in the case of participating minors, from their parents/guardians, prior to the performance of any study-specific procedures. In addition to the consent provided by their parents/guardians, all subjects aged 7 years and above also provided informed assent. The study protocol and the informed consent and assent were reviewed and approved by an institutional review board in Lebanon (Faculty of Medicine, American University of Beirut). This study has been registered at www.clinicaltrials.gov (NCT00661557).

2.2. Study objectives

The primary objective of this study was to evaluate the immunogenicity of the MenACWY–TT vaccine in terms of rSBA titers (serum bactericidal assay using rabbit complement) against each serogroup at 1 month post-vaccination in subjects who had either received one dose of the Men-PS vaccine between 30 and 42 months earlier or had not received any meningococcal vaccine within the past 10 years.

The secondary objectives of the study included the evaluation of the immunogenicity in terms of anti-PS and anti-TT antibody concentrations prior to and after MenACWY–TT vaccination and of the safety profile of the MenACWY–TT vaccine in subjects enrolled in the MPS and the noMPS groups.

2.3. Study subjects

Subjects were healthy males and females aged 4.5 to 34 years at the time of the MenACWY–TT vaccination, who had previously completed childhood vaccinations to the best of subject/parent/guardian knowledge. Subjects in the MPS group had received one dose of the Men-PS vaccine in a previous study, approximately 3 years earlier, and had not received any further meningococcal vaccine after completion of the previous study. Subjects in the noMPS group had not been vaccinated against meningococcal disease of any serogroup in the last 10 years.

Subjects were ineligible to participate in the study if they were immunosuppressed from any cause, had previously been vaccinated with a conjugate meningococcal vaccine at any time, or had received vaccination against tetanus within 30 days prior to administration of the study vaccine. Subjects with a history of meningococcal disease, chronic alcohol consumption, or drug abuse were also excluded. Moreover, subjects were ineligible if they had received immunoglobulins or blood products in the preceding 3 months, had a major congenital defect, serious illness, neurological or seizure disorder, or acute disease at the time of enrolment. Females of childbearing age were required to practice adequate contraception for 30 days prior to vaccination, have a negative pregnancy test at the time of vaccination, and to continue contraceptive precautions for 2 months after vaccination.

2.4. Vaccines

One dose of the MenACWY–TT vaccine (*Nimenrix*™, GlaxoSmithKline Biologicals SA, Rixensart, Belgium) contained 5 μg of each of the meningococcal serogroups A, C, W-135, and Y polysaccharides, conjugated to TT (~44 μg), and was supplied as a lyophilized pellet in a monodose vial for delivery of 0.5 ml after reconstitution with saline diluent. A single MenACWY–TT vaccine dose was administered intramuscularly in the non-dominant deltoid of all subjects.

In the previous study, subjects from the MPS group received one subcutaneous dose of the Men-PS vaccine (Mencevax® ACWY, GSK Biologicals, SA), which contained 50 μg of each of the meningococcal serogroups A, C, W-135, and Y polysaccharides.

2.5. Immunogenicity assessment

Immunogenicity was assessed by functional antibody responses against the four serogroups using a serum bactericidal activity (SBA) assay with baby rabbit serum as complement source (rSBA, cut-off 1:8).[27] rSBA-MenC titers ≥1:8 are considered to predict protection,[28] and in this study, this threshold was extended

to the other serogroups as well.[29] rSBA titers ≥1:128, a more conservative correlate of protection, were also computed.[30] Vaccine response was calculated and defined as an rSBA titer of at least 1:32 at 1 month after conjugate vaccination in subjects who were seronegative (rSBA titer <1:8) prior to conjugate vaccination, and as at least a four-fold increase in titer from pre-vaccination to 1 month post-vaccination in subjects who were seropositive prior to conjugate vaccination.

Antibody concentrations against meningococcal polysaccharides of all serogroups (anti-PS, cut-off 0.30 μg/ml)[31] and against TT (anti-TT, cut-off 0.10 IU/ml)[32] were determined by ELISA. The percentages of subjects with anti-PS concentrations ≥2.0 μg/ml as well as with anti-TT concentrations ≥1.0 IU/ml were also computed. Subjects from each group were sub-randomized into two subsets of 50% of the subjects to be tested for anti-PS antibody concentrations against either serogroups A and C or serogroups W-135 and Y. All the blood samples were tested at the central laboratories of GSK Biologicals.

2.6. Safety and reactogenicity assessment

Solicited local (pain, redness, and swelling) and general (fatigue, fever, gastrointestinal symptoms, and headache) adverse events (AEs) were recorded during a 4-day (days 0–3) follow-up period, while unsolicited AEs were recorded during a 31-day (days 0–30) follow-up period after MenACWY–TT administration. Symptom intensity was graded on a 0–3 scale, where symptoms of grade 3 intensity were defined as follows: site redness or swelling >50 mm, fever >39.5 °C, and all other AEs that prevented normal activities. The occurrence of new onset chronic illnesses (NOCIs) and serious adverse events (SAEs) were reported through 6 months after the vaccination. All solicited local AEs reported during the 4-day follow-up period were automatically considered to be related to vaccination. The relationship between vaccination and all other AEs was assessed by the investigator.

2.7. Statistical analyses

With 322 subjects from the previous study eligible to be enrolled in the MPS group and the 3:1 group ratio design, a sample size of 430 subjects was anticipated. It was expected that only a third of the subjects from the previous study would provide consent for the study and that 15% of enrolled subjects would be non-evaluable for the immunogenicity analysis. Hence 183 subjects were estimated in the MPS group and 61 in the noMPS group. A listing of subjects who were willing to participate was provided by the investigator. In order to ensure group comparability in age, this listing was used to select age-matched subjects for enrollment in the noMPS group.

The total vaccinated cohort (TVC) included all vaccinated subjects and was the primary cohort for the analysis of safety. Analysis of the solicited symptoms only included vaccinated subjects for doses with documented safety data (i.e. symptom sheet completed), for other safety analyses all vaccinated subjects were considered. The analyses of immunogenicity were performed on the according-to-protocol (ATP) cohort for immunogenicity, which included subjects who met all eligibility criteria and met no elimination criteria during the study, and for whom immunogenicity data for at least one antigen were available at month 1.

The percentages of participants with antibody titers or concentrations above the proposed cut-offs and of those with a vaccine response, as well as geometric mean antibody concentrations/titers (GMC/T) were calculated with exact 95% confidence intervals (95% CI) in each treatment group. Two groups were considered significantly different if the asymptotic standardized two-sided 95% CI for the difference between groups in percentages of participants with titers/concentrations above proposed cut-offs or with a vaccine response did not contain the value '0', or if the 95% CI for the GMT/GMC ratio between groups did not contain the value '1' (exploratory analyses). The GMT/GMC ratios were computed by an analysis of covariance model on the $\log_{10}$

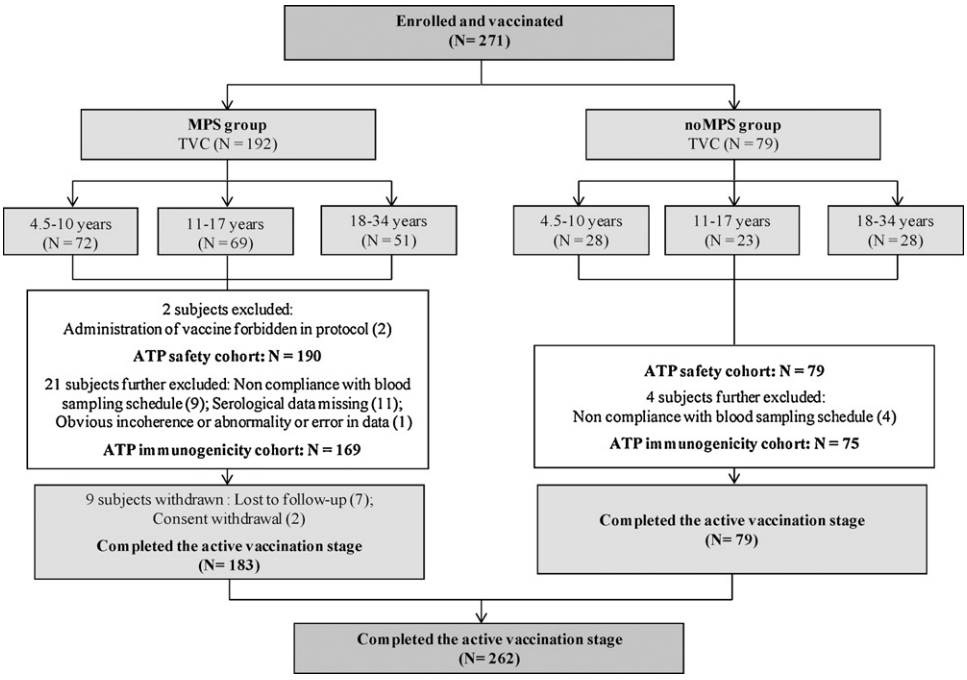

Figure 1. Number of enrolled subjects in each treatment group and reasons for eliminations from the study cohorts (MPS = subjects vaccinated with Men-PS in a previous study (NCT00227422); noMPS = subjects with no meningococcal vaccination within the past 10 years; TVC = total vaccinated cohort; ATP = according-to-protocol; *n* = number of subjects).

Table 1
Percentages of subjects with rSBA titers equal to or above cut-off values, and rSBA geometric mean titers (ATP cohort for immunogenicity)

Antibody	Group[a]	Timing[b]	*n*[c]	% ≥1:8 (95% CI)[d]	% ≥1:128 (95% CI)[d]	GMT (95% CI)[e]
rSBA-MenA	MPS	Month 0	160	100 (97.7–100)	100 (97.7–100)	1770.1 (1569.0–1996.9)
		Month 1	146	100 (97.5–100)	100 (97.5–100)	6868.8[f] (6044.9–7805.0)
	noMPS	Month 0	71	98.6 (92.4–100)	98.6 (92.4–100)	1103.8 (863.2–1411.5)
		Month 1	69	100 (94.8–100)	100 (94.8–100)	13014.9 (10722.2–15798.0)
rSBA-MenC	MPS	Month 0	165	75.2 (67.8–81.5)	59.4 (51.5–67.0)	116.6 (80.2–169.4)
		Month 1	169	100 (97.8–100)	97.0 (93.2–99.0)	1945.8[f] (1583.3–2391.1)
	noMPS	Month 0	70	47.1 (35.1–59.4)	37.1 (25.9–49.5)	30.1 (17.4–52.0)
		Month 1	75	100 (95.2–100)	100 (95.2–100)	5494.6 (4266.3–7076.5)
rSBA-MenW-135	MPS	Month 0	165	81.8 (75.1–87.4)	70.3 (62.7–77.2)	154.0 (114.0–208.1)
		Month 1	169	100 (97.8–100)	100 (97.8–100)	4635.7[f] (3942.5–5450.7)
	noMPS	Month 0	74	56.8 (44.7–68.2)	32.4 (22.0–44.3)	36.9 (22.7–59.9)
		Month 1	75	100 (95.2–100)	100 (95.2–100)	9078.0 (7087.7–11627.1)
rSBA-MenY	MPS	Month 0	167	94.6 (90.0–97.5)	89.8 (84.2–94.0)	555.2 (434.8–708.9)
		Month 1	169	100 (97.8–100)	100 (97.8–100)	7799.9[f] (6682.8–9103.6)
	noMPS	Month 0	74	81.1 (70.3–89.3)	74.3 (62.8–83.8)	170.1 (106.1–272.9)
		Month 1	75	100 (95.2–100)	100 (95.2–100)	13895.5 (11186.2–17260.9)

rSBA, serum bactericidal activity using rabbit complement; ATP, according-to-protocol; 95% CI, 95% confidence interval.
[a] MPS = subjects vaccinated with Men–PS in a previous study (NCT00227422); noMPS = subjects with no meningococcal vaccination within the past 10 years.
[b] Month 0 = pre-vaccination blood sample; Month 1 = blood sample taken at 1 month post-vaccination.
[c] *n* = number of subjects with available results.
[d] % = percentage of subjects with titer within the specified range.
[e] GMT = geometric mean antibody titer calculated on all subjects.
[f] Statistically significant lower value in the MPS group than in the noMPS group in an exploratory analysis.

transformation of the titers/concentrations using the pre-vaccination $\log_{10}$ transformation of the titers/concentrations, the age strata, and the vaccine group as covariates.

The incidence and intensity of each solicited and unsolicited AE were calculated with exact 95% CI. Two-sided asymptotic standardized tests were used to detect differences between the two groups for each solicited symptom (percentage of subjects with the symptom) and the associated two-sided *p*-values were calculated. NOCIs and SAEs were described in detail.

The statistical analyses were performed using the SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) and Proc StatXact 7.0.

3. Results

3.1. Study subjects

A total of 192 subjects from the previous study met the eligibility criteria and consented to enter this study; they were vaccinated in the MPS group (Figure 1). In the noMPS group, 79 age strata-matched subjects were enrolled and vaccinated. Of the 271 vaccinated participants, 262 subjects completed the visit at month 1. A total of 244 subjects were included in the ATP cohort for immunogenicity, and no subjects discontinued the study due to AEs or SAEs.

In the TVC, the mean age of the subjects across both vaccine groups at the time of enrolment was 14.1 years (range 4–33 years). Almost the entire population (99.3%) was of white-Arabic/North African heritage, with nearly equivalent proportions of males (51.3%) and females (48.7%). The demographic characteristics of the subjects were comparable in the two groups (data not shown).

3.2. Immunogenicity

3.2.1. Serum bactericidal activity after MenACWY–TT vaccination

The pre-vaccination rSBA GMTs were higher in the MPS group than in the noMPS group (Table 1). The rSBA GMTs increased from pre- to post-vaccination by 3.9- to 30.1-fold in the MPS group and by 11.8- to 246-fold in the noMPS group. One month after the MenACWY–TT vaccination, all the subjects in both groups were seropositive (rSBA titer ≥1:8) for the four serogroups, and the proportion of subjects with rSBA titers ≥1:128 ranged from 97.0% to 100% in the MPS group and was 100% for all four serogroups in the noMPS group. The vaccine response rates to each of the four serogroups ranged from 41.1% to 83.0% in the MPS group and from 76.9% to 97.3% in the noMPS group (Table 2).

Exploratory analyses did not detect any statistically significant differences between the MPS and the noMPS groups in terms of percentages of subjects with rSBA titers ≥1:8 and ≥1:128 at 1 month after the MenACWY–TT vaccination. However, the percentages of vaccine responders and the post-vaccination rSBA GMTs for the four serogroups were significantly lower in the MPS group than in the noMPS group.

3.2.2. Anti-PS antibody concentrations after MenACWY–TT vaccination

The pre-vaccination seropositivity rates in terms of percentages of subjects with anti-PS antibody concentrations ≥0.3 μg/ml were higher in the MPS group (88.2–97.6%) than in the noMPS group (11.4–36.8%) for the four serogroups. One month after vaccination, the seropositivity rates increased to at least 98.8%, and anti-PS concentrations ≥2.0 μg/ml were reached in 97.3% to 100% of the

Table 2
Percentages of subjects with rSBA vaccine response at 1 month post-vaccination (ATP immunogenicity cohort)

Antibody	Group[a]	*n*[b]	% Vaccine response (95% CI)[c]
rSBA-MenA	MPS	141	41.1[d] (32.9–49.7)
	noMPS	65	76.9 (64.8–86.5)
rSBA-MenC	MPS	165	66.7[d] (58.9–73.8)
	noMPS	70	97.1 (90.1–99.7)
rSBA-MenW-135	MPS	165	83.0[d] (76.4–88.4)
	noMPS	74	97.3 (90.6–99.7)
rSBA-MenY	MPS	167	74.9[d] (67.6–81.2)
	noMPS	74	95.9 (88.6–99.2)

rSBA, serum bactericidal activity using rabbit complement; ATP, according-to-protocol; 95% CI, 95% confidence interval.
[a] MPS = subjects vaccinated with Men–PS in a previous study (NCT00227422); noMPS = subjects with no meningococcal vaccination within the past 10 years.
[b] *n* = number of subjects with pre- and post-vaccination results available.
[c] % Vaccine response = percentage of subjects showing a vaccine response defined as follows: (1) for initially seronegative subjects: antibody titer ≥1:32 at 1 month post-vaccination; (2) for initially seropositive subjects: antibody titer at 1 month post-vaccination ≥4-fold the antibody titer at pre-vaccination.
[d] Statistically significant lower value in the MPS group than in the noMPS group in an exploratory analysis.

Table 3
Percentages of subjects with anti-PS concentrations equal to or above cut-off values, and geometric mean concentrations (ATP cohort for immunogenicity)

Antibody	Group[a]	Timing[b]	n[c]	% ≥0.3 μg/ml (95% CI)[d]	% ≥2.0 μg/ml (95% CI)[d]	GMC (95% CI)[e]
Anti-PSA	MPS	Month 0	82	93.9 (86.3–98.0)	84.1 (74.4–91.3)	9.38 (6.37–13.81)
		Month 1	82	98.8 (93.4–100)	98.8 (93.4–100)	79.15 (57.61–108.73)
	noMPS	Month 0	38	36.8 (21.8–54.0)	18.4 (7.7–34.3)	0.41 (0.24–0.70)
		Month 1	38	100 (90.7–100)	97.4 (86.2–99.9)	47.45 (30.48–73.85)
Anti-PSC	MPS	Month 0	82	97.6 (91.5–99.7)	74.4 (63.6–83.4)	6.46 (4.49–9.30)
		Month 1	82	100 (95.6–100)	100 (95.6–100)	31.19[f] (25.34–38.39)
	noMPS	Month 0	38	26.3 (13.4–43.1)	15.8 (6.0–31.3)	0.31 (0.20–0.47)
		Month 1	38	100 (90.7–100)	100 (90.7–100)	18.81 (12.92–27.38)
Anti-PSW-135	MPS	Month 0	76	88.2 (78.7–94.4)	59.2 (47.3–70.4)	3.40 (2.20–5.26)
		Month 1	85	100 (95.8–100)	98.8 (93.6–100)	25.10[f] (19.49–32.33)
	noMPS	Month 0	35	11.4 (3.2–26.7)	5.7 (0.7–19.2)	0.20 (0.14–0.29)
		Month 1	37	100 (90.5–100)	97.3 (85.8–99.9)	14.67 (9.25–23.28)
Anti-PSY	MPS	Month 0	85	91.8 (83.8–96.6)	65.9 (54.8–75.8)	4.05 (2.83–5.81)
		Month 1	86	100 (95.8–100)	98.8 (93.7–100)	29.98[f] (23.48–38.28)
	noMPS	Month 0	36	13.9 (4.7–29.5)	8.3 (1.8–22.5)	0.21 (0.15–0.29)
		Month 1	37	100 (90.5–100)	97.3 (85.8–99.9)	17.58 (11.89–25.99)

anti-PS, anti-polysaccharide antibodies; ATP, according-to-protocol; 95% CI, 95% confidence interval.
[a] MPS = subjects vaccinated with Men-PS in a previous study (NCT00227422); noMPS = subjects with no meningococcal vaccination within the past 10 years.
[b] Month 0 = pre-vaccination blood sample; Month 1 = blood sample taken at 1 month post-vaccination.
[c] n = number of subjects with available results.
[d] % = percentage of subjects with concentration within the specified range.
[e] GMC = geometric mean antibody concentration calculated on all subjects.
[f] Statistically significant higher value, adjusted for age strata, in the MPS group than in the noMPS group in an exploratory analysis.

subjects. Anti-PS GMCs for all serogroups increased after conjugate vaccination in both groups (Table 3).

Exploratory analyses did not detect any statistically significant differences between the MPS and the noMPS groups in terms of percentages of subjects with anti-PS concentrations ≥0.3 μg/ml and ≥2.0 μg/ml at 1 month after MenACWY–TT vaccination, but in contrast with rSBA GMTs, post-vaccination anti-PS GMCs, adjusted for age strata, were significantly higher in the MPS group compared to the noMPS group for serogroups C, W-135, and Y.

3.2.3. Anti-tetanus toxoid antibody concentrations

Prior to vaccination, there was a trend for lower anti-TT GMCs in the MPS group (0.718; 95% CI 0.561–0.919) compared to the noMPS group (1.515; 95% CI 1.032–2.223). One month after vaccination, seropositivity rates for anti-TT were 98.2% and 98.7% in the MPS and noMPS groups, respectively. Exploratory analyses showed that anti-TT GMCs, adjusted for age strata, were significantly lower in the MPS group (17.678; 95% CI 14.494–1.562) than in the noMPS group (41.600; 95% CI 31.587–54.787) at month 1.

3.3. Safety

In the TVC, solicited and unsolicited AEs were reported by 111 subjects (57.8%) in the MPS group and 41 subjects (51.9%) in the noMPS group during the 4-day post-vaccination period. Grade 3 symptoms were observed in 22 subjects (11.5%) in the MPS group compared to three subjects (3.8%) in the noMPS group. Compliance in returning the symptom sheets was 85.9% for subjects in the MPS group (n = 165) and 97.5% for subjects in the noMPS group (n = 77) for both local and general symptoms.

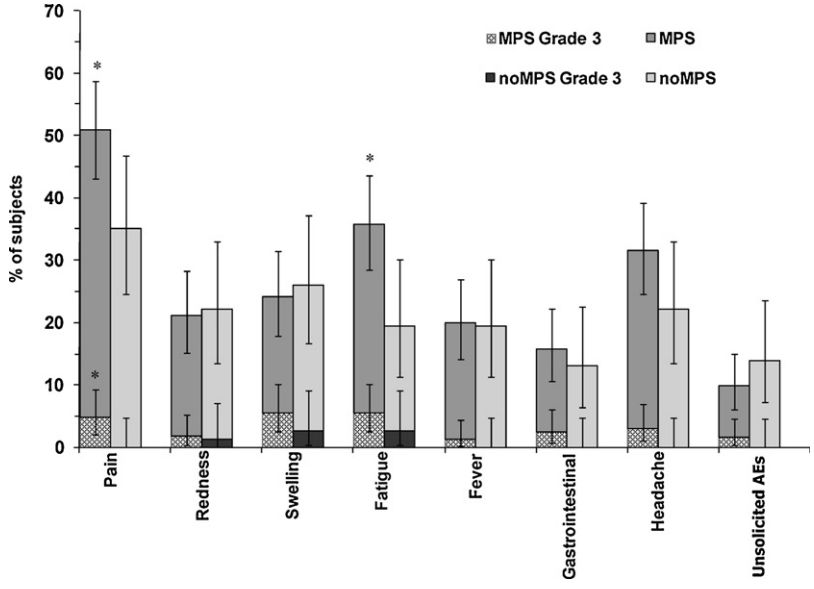

Figure 2. Incidence (with 95% confidence interval) of solicited local and general symptoms during the 4-day follow-up period after vaccination and of unsolicited symptoms during the 31-day follow-up period after vaccination (total vaccinated cohort) (error bars represent 95% confidence intervals; MPS = subjects vaccinated with Men-PS in a previous study (NCT00227422); noMPS = subjects with no meningococcal vaccination within the past 10 years; symptoms of grade 3 intensity were defined as follow: site redness or swelling >50 mm; fever >39.5 °C; all other adverse events that prevented normal activities.*p-value < 0.05).

At the MenACWY–TT injection site, pain was the most frequently reported solicited local AE in both groups and occurred in 84 subjects (50.9%) in the MPS group and 27 subjects (35.1%) in the noMPS group (Figure 2). Reported rates of redness and swelling were comparable in the MPS and noMPS groups (rates of redness were 21.2% and 22.1%, respectively, and rates of swelling were 24.2% and 26.0%, respectively). The most common solicited general AE in the MPS group was fatigue (59 subjects; 35.8%) and in the noMPS group was headache (17 subjects; 22.1%). Only a small percentage (5.5% at most) of subjects had solicited symptoms with grade 3 intensity after vaccination in both vaccine groups. Two subjects in the MPS group reported fever $>39.5\,^{\circ}C$, which was considered causally related to vaccination. Pain (any and grade 3), related fever, related headache, and fatigue (any and related) were reported more frequently in the MPS group than in the noMPS group (p-value < 0.05).

At least one unsolicited AE was reported within the 31-day follow-up period after the vaccine administration by 19 subjects (9.9%) in the MPS group and 11 subjects (13.9%) in the noMPS group (Figure 2). Throughout the entire study, one subject in the MPS group experienced an SAE (tendon rupture) and two subjects in the noMPS group each reported one NOCI (seasonal allergy and rhinitis). These events were not considered as causally related to MenACWY–TT vaccination. No deaths occurred during the study.

4. Discussion

The primary objective of this study was to evaluate the immunogenicity and safety of the MenACWY–TT vaccine when given to subjects previously vaccinated with a plain polysaccharide vaccine compared to subjects who had not received a meningococcal vaccine within the past 10 years, considering previous data on the potential hyporesponsiveness induced by meningococcal polysaccharide vaccines when subsequent doses are given.[33,34]

As expected, the pre-vaccination rSBA titers and anti-PS concentrations in the present study were higher in the subjects who had received the Men-PS vaccine in the previous study than in subjects without prior meningococcal polysaccharide vaccination within the past 10 years. One month after MenACWY–TT vaccination, rSBA GMTs had increased at least 3.9-fold, and at least 97.0% of subjects had rSBA titers $\geq$1:128 for all serogroups in both groups. The response in the subjects with no meningococcal vaccination within the past 10 years was consistent with that observed in previous studies conducted with the MenACWY–TT vaccine given in meningococcal vaccine-naïve subjects.[16–22] Moreover, rSBA GMTs measured in subjects initially immunized with the Men-PS vaccine for serogroups C, W-135, and Y were higher than those observed in the previous study (NCT00227422) at 1 month after administration of the Men-PS vaccine (data not shown).

Despite the increases observed in both groups, exploratory analyses showed that post-vaccination rSBA GMTs and vaccine response rates for all serogroups were significantly higher in the subjects with no meningococcal vaccination within the past 10 years than in the subjects with previous Men-PS vaccination. These results suggest that vaccination with a polysaccharide vaccine may reduce the response to a subsequent dose of either polysaccharide vaccine or conjugate vaccine.[14,25,26,34] Another possibility is that the high antibody concentrations noted prior to conjugate vaccination in the subjects with previous Men-PS administration may interfere with the meningococcal antigens and reduce the response to the meningococcal vaccine.[35] The lower vaccine response rates observed in the subjects with previous meningococcal vaccination may also be explained by the fact that when pre-vaccination titers are already high, it is more difficult to obtain a four-fold rise from pre- to post-vaccination, since the absolute post-vaccination titer required to meet the definition of vaccine response is much higher than 1:32.[36] Vaccine response rates are used to evaluate the immunogenicity of the meningococcal vaccines because they assess the ability of participants to respond to the vaccine regardless of their serostatus at pre-vaccination.[27,37] These findings of the present study are consistent with those of previous studies, in which lower immune responses, when measured by rSBA assay, to meningococcal conjugate vaccines were observed in subjects with previous vaccination with polysaccharide meningococcal vaccines compared with meningococcal vaccine-naïve subjects.[14,25,26,38,39]

In contrast, responses measured by ELISA were generally lower in the subjects without meningococcal vaccination within the past 10 years compared to the subjects with previous Men-PS vaccination. The reason for this discrepancy is not entirely understood, though several hypotheses have been suggested. The lower rSBA levels in subjects previously vaccinated with Men-PS may be explained by the fact that meningococcal polysaccharide vaccines induce a T-cell independent response, which leads primarily to the development of plasma cells as compared to memory cells. It has been suggested that upon re-exposure to polysaccharide, the plasma cells are stimulated but not replenished, resulting in an overall depletion of the memory cell pool. Subsequent administration of a conjugate vaccine may be able to overcome this depletion to a certain extent.[34] However, the ELISA assay only measures total antibody concentration, and does not evaluate the functional capacity of those antibodies. Although previous priming with polysaccharide vaccine may result in overall higher antibody concentrations, the bactericidal activity induced after the conjugate vaccine may be reduced.

A side observation of this study is that higher anti-TT concentrations were observed in the group of subjects with no previous meningococcal vaccination compared to the group of subjects who received the Men-PS vaccine in the previous study, both prior to vaccination and at 1 month post-vaccination with MenACWY–TT. Post-vaccination anti-TT differences are difficult to interpret because of the possible selection bias introduced into the study by the lack of randomization and the smaller sample size in the control group. The difference in pre-existing TT immunity between the two groups, also reflected by post-vaccination anti-TT response, is also a confounding factor to the understanding of the different immunogenicity results (anti-PS and rSBA) between the two groups.

MenACWY–TT has previously been shown to be well-tolerated in toddlers, children, and adults.[16–22] In this study, a higher incidence of some of the solicited symptoms was observed in the subjects with prior polysaccharide vaccination. This is in contrast with the immunogenicity results, as higher immunogenicity was obtained in the subjects without prior polysaccharide vaccination. Several factors may contribute to reactogenicity, including preexisting immunity to the meningococcal and TT antigens. Also the sample size of the study was limited, the subjects in the MPS group were less compliant in returning their symptom sheets, and the observed differences may be non-relevant study findings. Nonetheless, both vaccination regimens were associated with an acceptable safety profile, with each grade 3 solicited symptom reported in no more than 5.5% of recipients in each treatment group.

The study was limited by its open design, as all subjects received the same study vaccine, and by the numerous exploratory statistical comparisons performed with no adjustment for multiplicity. It was recognized that any observed differences might be due to chance and therefore must be interpreted within the context of clinical relevance. Another limitation of this study comes from the possible selection bias introduced into the study by the lack of randomization and the smaller sample size in the

control group. Indeed, the two groups could not be randomized as the subjects of the MPS group were recruited amongst subjects vaccinated in a previous study with a meningococcal polysaccharide vaccine. This was mitigated by enrolling the subjects of the control group amongst age-matched subjects coming from the same region. Finally, longer follow-up periods could be considered in the future, as differences in antibody titers immediately post-vaccination may not translate into differences in long-term antibody persistence.

In conclusion, these data support revaccination with one dose of the MenACWY–TT conjugate vaccine following previous vaccination with the Men-PS vaccine if sustained protection against invasive meningococcal disease is needed. MenACWY–TT induced a robust response in subjects previously vaccinated with a plain polysaccharide vaccine even though bactericidal antibody titers were lower than in subjects with no meningococcal vaccination in the past 10 years. These results suggest that the MenACWY–TT vaccine could be considered for implementation in vaccination programs irrespective of the pre-vaccination status with a polysaccharide vaccine.

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